Highly efficient large-scale lentiviral vector concentration by tandem tangential flow filtration.

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Public Summary:
Large-scale lentiviral vector (LV) concentration can be inefficient and time consuming, often involving multiple rounds of filtration and centrifugation. This report describes a simpler method using two tangential flow filtration (TFF) steps to concentrate liter-scale volumes of LV supernatant, achieving in excess of 2000-fold concentration in less than 3h with very high recovery (>97%). Large volumes of LV supernatant can be produced easily through the use of multi-layer flasks, each having 1720cm(2) surface area and producing approximately 560mL of supernatant per flask. Combining the use of such flasks and TFF greatly simplifies large-scale production of LV. As a demonstration, the method is used to produce a very high titer LV (>10(10)TU/mL) and transduce primary human CD34+ hematopoietic stem/progenitor cells at high final vector concentrations with no overt toxicity. A complex LV (STEMCCA) for induced pluripotent stem cell generation is also concentrated from low initial titer and used to transduce and reprogram primary human fibroblasts with no overt toxicity. Additionally, a generalized and simple multiplexed real-time PCR assay is described for lentiviral vector titer and copy number determination.

Scientific Abstract:
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